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Bio-active Me tabolites from the Brown Alga *Dictoyota dichotoma.*

Seham E. Abu Ahmed[a], Fawzia Z. El-Ablack[b], Shayma H. Ahmed[b], Ahmed A. Alotaibi[c], Walied M. Alarif[d], and Seif-Eldin N. Ayyad[b,*].

[a]Botany Department, Faculty of Science, Damietta University, Damietta 34517, Egypt.
[b]Chemistry Department, Faculty of Science, Damietta University, Damietta 34517, Egypt.
[c]Department of Chemistry, Faculty of Science, University of Ha'il, P.O. Box 2440, Ha'il 81451, Saudi Arabia.
[d]Department of Marine Chemistry, Faculty of Marine Sciences, King Abdulaziz University, PO. Box 80207, Jeddah 21589, Saudi Arabia.

ABSTRACT

Air dried algal materials of *Dictyota dichotoma* were extracted with a mixture of organic solvents, followed by purification on silica gel, led to isolation of six pure metabolites. After establishing the chemical structures, their antimicrobial, cytotoxicity and antioxidant activities were assayed. Six compounds were obtained. Compounds **1** and **2** are significantly potent against *Klebsiella pneumonia* and even higher than that of the positive control, penicillin G. Compounds **3**, **5** and **6** at a concentration of 50µg/ml showed comparable effect with that of chloramphenicol. Compound **5** showed significant activity against *Candida albicans* (10-16 mm) and *Fusarium oxysporium* (15- 19 mm). Dictyol (**6**) and the total extract showed strong cytotoxic activity against two cancer cell lines (MCF-7 and HepG-2). The total extract displayed antioxidant effect. Both total extract and the isolated compounds showed potent antimicrobial effects compared with several reference antibiotics. The measured activities of the total extract are mainly higher than those of the pure compounds, which could be due to other effective compound(s) or synergistic action between the isolated compounds.

Keywords: Red Sea, Marine organisms, Cytotoxicity, Terpenoids, Antimicrobial.

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Corresponding author

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INTRODUCTION

Among marine organisms, macro-algae are highly productive of diversity of important natural compounds. Majority of them showed economical and medicinal applications [1]. They are considered as a vital source of fibers, minerals, vitamins, pigments, steroids, lectins, halogenated compounds, polyketides, and polysaccharides. Those compounds displayed essential biological effects, embracing, anti-inflammatory, antioxidant, anticancer and antimicrobial properties [2].

Species of the brown algal genus *Dictyota* are widespread seaweeds in tropical marine habitats, particularly, Atlantic and Indian Oceans [3]. The *D. dichotoma* produces three main types of diterpenes (xenicanes, extended sesquiterpenes, and dolabellanes). The production of these metabolites depends on time and area of collection [4]. These brown algae are considered as a source of diverse metabolites, which exhibited biological effect such as antimicrobial, antiviral, antifungal, antitumor and cytotoxic effects [5-7].

A marine brown macro-alga, identified as *D. dichotoma*, collected from Salman gulf north of Jeddah, was investigated. The total extract (Pet.-ether: Methylene chloride: Methanol (1:1:1, v/v)) had been fractionated using different chromatographic techniques to give six metabolites; pachydictyol A (1), isopachydictyol A (2), amijiol acetate (3) 8β-hydroxypachydictyol (4), amijiol (5) and dictyol C (6) (Figure 1). The total extract and the isolated compounds have been evaluated for their cytotoxicity, employing two cancer cells HepG2, and MCF-7, and also for their antimicrobial and antioxidant activities.

MATERIALS AND METHODS

General

Silica Gel 60 mesh (Fluka) used as chromatographic material for column chromatography. The thin layer chromatography (TLC): Silica gel 60 mech F 254 (Merck). Preparative Thin Layer Chromatography (PTLC): Pre-coated TLC- glass plates Sil G-25UV 254 (MACHEREY_NAGEL). Visualization of TLC was performed with UV light and *p*-anisaldehyde as spray reagent.

Chemicals: Dimethyl sulfoxide (DMSO), MTT 3- (4,5-dimethylthiazol-2-yl) -2,5-diphenyltetrazolium bromide dye were purchased from Sigma (St. Louis, Mo., USA). Fetal Bovine serum, DMEM, RPMI-1640, HEPES buffer solution, L-glutamine, gentamycin and 0.25% Trypsin-EDTA were purchased from Lonza. Crystal violet stain (1%): It composed of 0.5% (w/v) crystal violet and 50% methanol then made up to volume with double distilled H_2O and filtered through a Whatmann No.1 filter paper.

Extraction of *Dictyota dichotoma*

A specimen of *D. dichotoma* collected in May. 2018 from Al-Shuaiba area (20°42'52.36"N; 39°29'20.74"E), Jeddah, Saudi Arabia, was air-dried to give 260.0 g extract, which was macerated with mixture of Pet.-ether: Methylene chloride: Methanol (1:1:1, v/v)). The residue (24.0 g) obtained after evaporation of the solvent was fractionated by a neutral aluminum oxide column using gradient elution (*n*-hexane/chloroform).

Isolation of compounds 1-6

Fraction A eluted with pet.-ether- chloroform (9.7: 0.3, v/v) was purified using PTLC and solvent system pet.-ether: chloroform (8.7:1.3). A dark blue band R_f = 0.5 visualized up on spraying with *p*-anisaldehyde was scraped and the silica was filtered to yield 13 mg of oily colorless material **(1** and **2).** Fraction B eluted with solvent system pet.-ether- chloroform (9.2:1.8), was purified using PTLC and pet.-ether: chloroform (8:2). A purple band R_f= 0.48 visualized up on spraying with *p*-anisaldehyde gave pure compound **3** was obtained. Fraction C eluted with pet.-ether- chloroform (85:15) was purified with PTLC and pet.-ether: chloroform (75:25). A violet band found at PTLC R_f= 0.29 visualized up on spraying with *p*-anisaldehyde. The band was scraped and the silica was filtered, a pure compound **4** was obtained. Fraction D eluted with pet.-ether- chloroform (8:2) was purified with PTLC and petroleum ether: chloroform (7:3). A brown band found at PTLC R_f = 0.3 visualized up on spraying reagent *p*-anisaldehyde. The band yielded a pure compound **5**. Fraction E eluted with pet.-ether-chloroform (65:35) was purified with PTLC and petroleum ether: chloroform (65:35).

The violet band found at R_f = 0.14 visualized up on spraying with *p*-anisaldehyde gave a pure compound **6** was obtained.

Characterization of the isolated compounds.

All compounds were identified by comparison with the corresponding published data.

Pachydictyol A (1):
GCMS (70 ev) m/z (rel.int) 288 (40) [M^+][$C_{20}H_{32}O$], 270 (6) [M^+-H_2O], 255 (9) [M^+-CH_3-H_2O], 159 (100), 107 (65), 105 (75),82(55), 69 (62). IR (film) 3490, 2982, 1632 cm^{-1}; ^1H, ^{13}C NMR ($CDCl_3$) □ ppm see table1.

Isopachydictyol A (2)
GCMS (70 ev) m/z (rel.int) 288 (40) [M^+][$C_{20}H_{32}O$], 270 (6) [M^+-H_2O], 255 (9) [M^+-CH_3-H_2O], 159 (100), 107 (65), 105 (75),82(55), 69 (62). IR (film) 3490, 2982, 1632 cm^{-1}; ^1H, ^{13}C NMR ($CDCl_3$) □ ppm see table1.

Amijiol acetate (3)
Colorless crystal (3 mg); EIMS (70 eV) m/z (rel.int) 346 (15) [M^+, $C_{22}H_{34}O_3$], 286 (17) [M^+-CH_3COOH], 149 (100), 135 (30), 107 (55), 93 (40); IR (film) 3585, 2950, 1735, cm^{-1}; ^1H, ^{13}C NMR ($CDCl_3$) □ ppm see table1.

8β-hydroxypachydictyol A (4)
Yellow oil (26 mg); EIMS (70 eV) m/z (rel.int) 304 (10) [M^+, $C_{20}H_{32}O_2$], 286 (15) [M^+-H_2O], 268 (5) [M^+-2 H_2O], 145 (45), 105 (59), 82 (85), 69 (100); IR (film) 3460, 2935, 1660, 1455, 1390 cm^{-1}; ^1H, ^{13}C NMR ($CDCl_3$) □ ppm see table2.

Amijiol (5)
Colorless crystal (8 mg); EIMS (70 eV) m/z (rel.int) 304 (10) [M^+ , $C_{20}H_{32}O_2$], 286 (15) [M^+-H_2O], 243 (20) [M^+-H_2O+C_3H_7], 149 (97) [M^+-$C_{11}H_{17}$]; IR (film) 3360, 2950, 1665, 885 cm^{-1}; ^1H, ^{13}C NMR ($CDCl_3$) □ ppm see table2.

Dictyol C (6)
White solid (8 mg); EIMS (70 eV) m/z (rel.int) 306 (10) [M^+, $C_{20}H_{34}O_2$], 288 (17) [M^+-H_2O], 270 [M^+-2H_2O],159(93) [M^+-2H_2O-C_8H_{15}], 69 (97) [M^+-2H_2O-$C_{15}H_{21}$]; IR (film) 3465, 2850, 1700, 1505 cm^{-1}; ^1H, ^{13}C NMR ($CDCl_3$) □ ppm see table2.

Biological evaluation of the isolated compounds

Antibacterial assay

The bacteria were grown on a medium specially designed for this work [8], which consists of glucose, 5 g; peptone, 5 g; sodium chloride, 5 g; beef extract, 3 g; agar, 15 g and distilled water to 1 L. 15 ml of aliquots of this agar were introduced into sterile Petri-dishes and seeded with 0.1 ml of an 18h.-old nutrient broth culture of tested bacterium. Control experiments were carried out under similar conditions by using norfloxacin, ciprofloxacin, chloramphenicol and penicillin G for antibacterial activity [9], employing *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosae* and *Klebsiella pneumonia.* These strains were obtained from the laboratory of Bacteriology (Faculty of Science - Damietta University, Egypt). Control experiments were carried out under similar conditions by using norfloxacin, ciprofloxacin, chloramphenicol and penicillin G for antibacterial activity.

Antifungal assay

Algal material was screened for the presence of antifungal activity by the method of Calvo *et al.* (1986) using different species of fungi: *Aspergillus niger, A. fumigatus, Fusarium oxysporium- pisi, Penicilliumnotatum* and *Candida albicans* as tested fungi [10]. These fungi were obtained from the laboratory of Mycology, Damietta Faculty of Science, Damietta University, Egypt.

To prepare the inoculum, a portion of each fungus to be tested was inoculated into 10 ml sterile water (saline solution). One ml of the suspension was transferred to Petri-dishes with DOX Agar media which consisted of (sucrose, 30 g; KH2PO4, 0.5 g; NaNO3, 3 g; MgSO4.7H2O, 0.5 g; KCl, 1 g; $FeSO_4.7H_2O$, traces; agar,

15 g and distilled water to 1 L.) and spread by gentle inversion to obtain uniform inoculum. The excess of inoculum is removed with a sterile pipette.

Testing procedures

Using a sterile cork borer, 10 mm "wells" were cut from the plate. 0.3 ml of algal extract was introduced into each well. For the control, 0.3 ml of DMSO was used. Inhibition zones were read one day after incubation at 37°C for bacteria and after 5-6 days at 28°C for fungi. Each assay was repeated two times and the mean values were recorded.

Cytotoxicity assay

Mammalian cell lines: HepG-2 cells (Human hepatocellular carcinoma) and MCF-7 cells (human breast carcinoma) were obtained from VACSERA (Egyptian company for production of vaccines, sera and drugs) Tissue Culture Unit. The cytotoxicity assay was performed according to Mosmann (1983) [11]. Cells (1 $\times 10^5$/well) were plated in 100 l of medium/well in 96-well plates (Costar Corning, Rochester, NY). After 48 hours incubation the cell reaches the confluence. Then, cells were incubated in the presence of various concentrations of the samples in 0.1% DMSO for 48h at 37°C. After removal of the sample solution and washing with phosphate-buffered saline (pH 7.4), 20µl/well (5mg/ml) of 0.5% 3-(4, 5- dimethyl-2-thiazolyl)-2, 5-diphenyl-- tetrazolium bromide cells (MTT) phosphate- buffered saline solution was added. After 4h incubation, 0.04M HCl/ isopropanol were added. Viable cells were determined by the absorbance at 570nm. Measurements were performed and the concentration required for a 50% inhibition of viability (IC50) was determined graphically. The absorbance at 570 nm was measured with a UV- Spectrophotometer using wells without sample containing cells as blanks. The effect of the samples on the proliferation of human breast cancer cells was expressed as the % cell viability, using the following formula: % cell viability = A570 of t treated cells / A570 of control cells × 100%.

Antioxidant assay

The antioxidant activity of extract was determined at the Regional Center for Mycology and Biotechnology (RCMB) at Al- Azhar University *by* the DPPH free radical scavenging assay in triplicate and average values were considered.

DPPH Radical Scavenging Activity:

The percentage inhibition (PI) of the DPPH radical was calculated according to the formula:

$$PI = [\{(AC- AT)/ AC\} \times 100] \quad (1)$$

Where AC = Absorbance of the control at t = 0 min and AT = absorbance of the sample+DPPH at t = 16 min [12].

Statistical analysis

Results are presented as the mean ± standard deviation (SD (of three replicates (n=3). The statistical analyses were carried out using SPSS (version 22). Data obtained were analyzed statistically to determine the degree of significance using a two-way analysis of variance (ANOVA) at probability level $p \leq 0.05$.

RESULTS AND DISCUSSION

A marine brown macro-alga, identified as *Dicoyota dichotoma*, collected from Al-Shuaiba area, Jeddah, was investigated. The total extract (Pet.-ether: Methylene chloride: Methanol (1:1:1, v/v)) had been fractionated using different chromatographic techniques to give six metabolites; pachydictyol A (**1**), isopachydictyol A (**2**), amijiol acetate (**3**) 8β-hydroxypachydictyol (**4**), amijiol (**5**) and dictyol C (**6**) (Figure 1). The total extract and the isolated compounds have been evaluated for their cytotoxicity, employing two cancer cells HepG2, and MCF-7, and also for their antimicrobial and antioxidant activities.

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Chemistry

Six diterpenoid derivatives were isolated from *Dictyota dichotoma* (Figure 1). Compound **1**was isolated as colorless oil. The ^{1}H and ^{13}C NMR data along with MS and IR spectral data (*cf. exp.*) of compound **1** were identical as reported for pachydictyol A [13].Compound **2** was isolated as colorless oil. The ^{1}H and ^{13}C NMR data along with MS and IR spectral data (*cf. exp.*) of compound **2** were identical as reported for isopachydictyol B [14].Compound **3** was isolated as colorless crystals. The ^{1}H and ^{13}C NMR data along with MS and IR spectral data (*cf. exp.*) of compound **3** were identical as reported for amijiol acetate [15].Compound **4**was isolated as yellow oil. The ^{1}H and ^{13}C NMR data along with MS and IR spectral data (*cf. exp.*) of compound **4** were identical as reported for 8β-hydroxypachidictyol A [16]. Compound **5** was isolated as colorless crystals. The ^{1}H and ^{13}C NMR data along with MS and IR spectral data (*cf. exp.*) of compound **5** were identical as reported for amijiol [18].Compound **6** was isolated as white solid. The ^{1}H and ^{13}C NMR data along with MS and IR spectral data (*cf. exp.*) of compound **6** were identical as reported for dictyol C [17].

Figure 1. Compounds isolated from *Dictyota dichotoma*

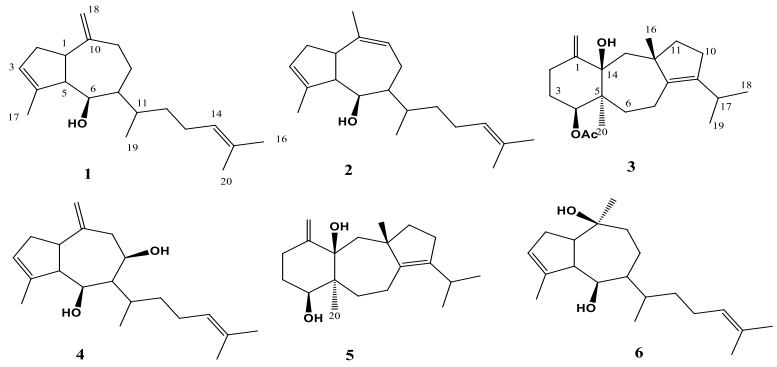

Table 1. ^{1}H, ^{13}C NMR of compounds 1-3

	1		2		3	
No	**δ ^{1}H (*J* in Hz)**	**^{13}C**	**δ ^{1}H (*J* in Hz)**	**^{13}C**	**δ ^{1}H (*J* in Hz)**	**^{13}C**
1	2.06 (m)	46.6 (d)	2.02 (m)	46.4 (d)	-	151.6 (s)
2	1.30 (m), 1.05 (m)	34.2 (t)	1.32 (m), 1.02(m)	35.2 (t)	2.16 (m) 2.7 (m)	27.8 (t)
3	5.37 (brs)	124.5 (d)	5.37 (brs)	124.0 (d)	1.75 (dddd, 12.0, 4.2, 3.0, 2.4), 1.83 (dddd, 12.0, 12.0, 5.4, 3.0)	27.1 (t)
4	-	141.2 (s)	-	142.2 (s)	4.79 (t, 2.4)	83.0 (d)
5	2.67 (m)	60.2 (d)	2.65 (m)	57.2 (d)	-	44.5 (s)
6	3.95 (m)	75.2 (d)	3.95 (m)	74.8 (d)	1.6 (ddd, 15.0, 6.6, 1.2), 2.14 (m)	27.6 (t)
7	1.35 (m)	47.5 (d)	1.32 (m)	46.5 (d)	2.12 (m), 2.45 (ddd, 16.8, 12.6, 6.6)	22.0 (t)
8	1.78 (m), 1.51 (m)	23.7 (t)	2.30 (m), 1.92 (m)	24.7 (t)	-	139.6 (s)
9	1.76 (m), 1.54 (m)	40.4 (t)	5.53 (brd, 9.0)	126.2 (d)	-	138.0 (s)
10	-	152.8 (s)	-	138.3 (s)	2.08 (ddd, 12.6, 3.6, 1.2), 2.63 (m)	26.9 (t)
11	1.56 (m)	35.3 (d)	1.52 (m)	33.4 (d)	1.54 (ddd, 12.0, 9.0, 9.0), 1.66(m)	42.7 (t)
12	2.16 (m), 1.63 (m)	35.6 (t)	2.13 (m), 1.60 (m)	34.9 (t)	-	51.0 (s)
13	1.62(m), 1.57(m)	25.4 (t)	1.64 (m), 1.55 (m)	25.2 (t)	1.69 (d, 14.4), 1.86 (dd, 14.4, 2.4)	46.0 (t)
14	5.15 (brt, 7.10)	124.3 (d)	5.15 (brt, 7.1)	124.4 (d)	3.64 (d, 2.4)	79.0 (s)
15	-	131.4 (s)	-	131.8 (s)	4.84 (brs), 4.9 (brs)	109.8 (t)
16	1.64 (d, 0.90)	25.3 (q)	1.64 (d, 0.9)	25.9 (q)	1.39 (s)	26.6 (q)
17	1.82 (td, 2.0, 1.2)	15.7 (q)	1.82 (td, 2.5, 1.5)	16.6 (q)	2.63 (h, 6.6)	27.0 (d)
18	4.72 (brs), 4.73 (brs)	107.4 (t)	1.75 (d, 1.5)	23.0 (q)	0.96 (d, 6.6)	21.1 (q)
19	0.94 (d, 6.0)	17.3 (q)	0.94 (d, 6.6)	17.2 (q)	0.90 (d, 6.6)	20.6 (q)
20	1.65 (s)	17.5 (q)	1.65 (s)	17.9 (q)	0.86 (s)	17.4 (q)
OAc					- 2.13 (s)	169.4 (s) 21.7 (q)

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Table 2. ^{1}H, ^{13}C NMR of compounds 4-6

	4		5		6	
No	**δ ^{1}H (*J* in Hz)**	**^{13}C**	**δ ^{1}H (*J* in Hz)**	**^{13}C**	**δ ^{1}H (*J* in Hz)**	**^{13}C**
1	2.75 (m)	45.0 (d)	-	152.5 (s)	2.10 (m)	49.2 (d)
2	2.17 (m), 2.45 (m)	33.0 (t)	2.50 (m), 2.90 (ddd, 15.0, 12.60, 6.00)	30.8 (t)	1.30 (m), 1.01 (m)	33.0 (t)
3	5.37 (brs)	121.9 (d)	1.75 (m), 1.84 (dddd, 14.4, 14.4, 4.8, 3.0)	27.0 (t)	5.30 (brs)	123.1 (d)
4	-	139.8 (s)	3.43 (dd, 8.40, 1.80)	81.0 (d)	-	142.4 (s)
5	2.57 (m)	56.0 (d)	-	44.0 (s)	2.78 (dd, 7.8, 6.0)	52.5 (d)
6	4.10 (m)	70.0 (d)	1.40 (m), 2.15 (m)	28.0 (t)	3.85 (dd, 7.8, 3.6)	74.6 (d)
7	1.57 (m)	49.0 (d)	2.25 (m), 2.53 (ddd, 15.80, 13.20, 8.40)	22.0 (t)	1.30 (m)	50.0 (d)
8	4.13 (m)	66.3 (d)	-	139.0 (s)	1.53 (m), 1.70 (m)	29.5 (t)
9	2.32 (dd, 4.20, 15.0), 3.02 (br dd, 3.30, 15.0)	43.0 (t)	-	138.6 (s)	1.75 (m), 1.55 (m)	46.4 (t)
10	-	145.0 (s)	2.18 (m), 2.83 (ddd, 14.40, 14.40, 5.40)	27.6 (t)	-	72.7 (s)
11	1.91 (m)	31.3 (d)	1.58 (m), 1.65 (m)	43.0 (t)	1.54 (m)	34.2 (d)
12	1.30 (m), 1.70 (m)	33.5 (t)	-	50.5 (s)	2.21 (m), 1.59 (m)	34.9 (t)
13	1.98 (m), 2.80 (m)	24.0 (t)	1.50 (d, 14.40), 1.64 (d, 14.40)	47.6 (t)	1.66 (m), 1.61 (m)	25.6 (t)
14	5.15 (brt, 7.4)	122.0 (d)	-	80.8 (s)	5.10 (brt, 7.1)	124.5 (d)
15	-	129.7 (s)	4.85 (brs), 4.90 (brs)	109.3 (t)	-	131.9 (s)
16	1.65 (s)	23.5 (q)	1.34 (s)	26.8 (q)	1.70 (d, 0.9)	25.4 (q)
17	1.80 (br s)	12.7 (q)	2.66 (h, 6.60)	26.5 (d)	1.83 (dd, 2.0, 1.2)	16.1 (q)
18	4.90(brs), 4.94(brs)	109.9 (t)	0.97 (d, 6.60)	21.5 (q)	1.18 (s)	30.3 (q)
19	1.15 (d, 7.0)	16.3 (q)	0.90 (d, 6.60)	20.1 (q)	0.98 (d, 6.6)	17.7 (q)
20	1.60 (s)	15.0 (q)	0.77 (s)	17.5 (q)	1.63 (s)	17.9 (q)

Biology

The bacterial infection causes a high rate of mortality in the human population and aquaculture organisms [18]. In the current results apparently, antibacterial inhibition zones have been observed due to the application of the extracts of *D. dichotoma* against the tested bacteria ranged from 1 to 15 mm. These results are by previously published by AL–Saif *et al.* (2014) and Kausalya and Narasimha (2015) which stated that macroalgae produce a wide variety of chemically active metabolites that have a broad range of biological activities [19, 20]. The data represented in table 1 indicated that antibacterial activities of all compounds increased linearly with an increase in the concentration of extracts (mg/ml). These results are in agreement with Abou-Dobara et al (2019) and which found that the effect against gram-positive bacteria increased by increasing the concentration (Table 3) [21]. Total extract concentrations of 1, 10, 25 and 50 mg/ml inhibited the growth of all microorganisms and exhibited a broad spectrum of antibacterial activity against Gram-positive bacteria *Staphylococcus aureus* (10 mm) as the same as Gram-negative bacteria *Pseudomonas aeruginosa* (10 mm). This result was agreed with Tajbakhsh *et al.* (2011) and Kim *et al.* (2007) which found that the crude extracts of the brown alga *Sargassum oligocystum* were examined and give the same results on gram-positive as well as gram-negative bacteria [9, 22]. It means that *D. dichotoma* contains specific natural compounds that display a strong potential to penetrate the complex structure of the Gram (+) and Gram (-) bacterial cells wall [23]. The concentration of 50 mg/ml showed the highest effect against all bacterial species. Compounds **4**, **5** and **6** were completely devoid of any activity against *P. aeruginosa*. On the other hand, the degree of **1**, **2** was practically zero against *S. aureus* and traces activity against *E. coli* while recorded the highest activity against *P. aeruginosa*. In contrast, Salvador *et al.* (2007) found that the extracts of *D. dichotoma* inactive against *E. coli* but with trace activity against *S. aureus* [24].

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Table 3. Antibacterial activities of the isolated compounds of *D. dichotoma*

Compound	µg/ml	width of inhibition zone (mm) ± SD			
		S. areus	*E. coli*	*P. aeruginosae*	*K. pneumonia*
Total Extract	1	1±0.06	1±0	5±0.01	2±0
	10	4.7±0.35	2±0	6±0.03	4±0.06
	25	6±0.06	3±0	9±0.03	5±0.01
	50	10±0.06	4±0.06	10±0.01	8±0.06
Pachydictyol A, Isopachydictyol A	1	0	0	8±0.03	3±0
	10	0	2±0	10±0.01	4±0.06
	25	0	3±0	11±0.06	5±0.03
	50	0	4±0	15±0.06	8±0.06
Amijiol acetate	1	0	3±0	2±0	8±0.06
	10	0	4±0	3±0	9±0.06
	25	5±0.01	5±0.06	5±0	10±0.06
	50	9±0.03	6±0.06	6±0.03	12±0.03
8β-hydroxypachydictyol A	1	0	0	0	2±0
	10	0	0	0	4±0
	25	7±0.06	3±0.06	0	5±0.01
	50	8±0	4±0.06	0	7±0
Amijiol	1	2±0	0	0	5±0
	10	4±0.01	2±0	0	6±0
	25	5±0.06	5±0.06	0	8±0.06
	50	7±0.01	7±0.03	0	12±0.06
Dictyol C	1	0	4±0.01	0	3±0.06
	10	2±0.06	5±0.06	0	5±0.01
	25	9±0.03	7±0.06	0	6±0
	50	10±0.06	8±0.03	0	12±0.06

The effect of the main factors (concentrations and compounds) and their interaction on antibacterial activity of *D. dichotoma* extracts were very highly significant ($p<0.05$) (Table 4). The effect of a concentration was stronger (with a higher F ratio) than that of a compound for all bacterial species. Standard drugs were mainly active against Gram-positive bacteria (*S. aureus*). But in *E. coli, P. aeruginosa and K. pneumonia* were more resistant as compared with *S. aureus*. **1** and **2** displayed effect against *K. pneumonia* higher than that of penicillin G by at least two times; this effect raises with concentration. **3**, **5** and **6** at a concentration of 50 mg/ml showed the same effect of the same concentration of chloramphenicol (12 mm) (Table 3 and 5).

Table 4. Two-way ANOVA showing the effect of the main factors (concentrations and compounds) and their interaction on bacterial inhibition zone diameter of *D. dichotoma* extracts

variables and source of variation	*df*	*F*	*p*
Conc	3	27059.6	.000
Bacteria	3	5126.1	.000
Compound	5	2708.4	.000
Conc * bacteria	9	1199.6	.000
Conc * compound	15	266.2	.000
bacteria * compound	15	7919.6	.000
Conc * bacteria * compound	45	504.1	.000

Table 5. Antibacterial activity of standard drugs against bacterial test organism

Standards	mg/ml	width of inhibition zone (mm) ± SD			
		S. areus	*E. coli*	*P. aeruginosae*	*K. pneumoniae*
Norfloxacin	1	13±0.06	10±0.06	10±0.06	11±0.06
	10	13±0.03	14±0.01	11±0.03	12±0.03
	25	14±0.06	14±0.06	11±0.06	13±0.01
	50	14±0.03	14±0.06	11±0.01	13±0.06
Ciprofloxacin	1	13±0.01	14±0.03	12±0.03	13±0.03
	10	15±0.03	14±0.35	14±0.06	14±0.01
	25	15±0.01	14±0.03	14±0.03	15±0.03
	50	15±0.06	14±0.06	14±0.06	15±0.06

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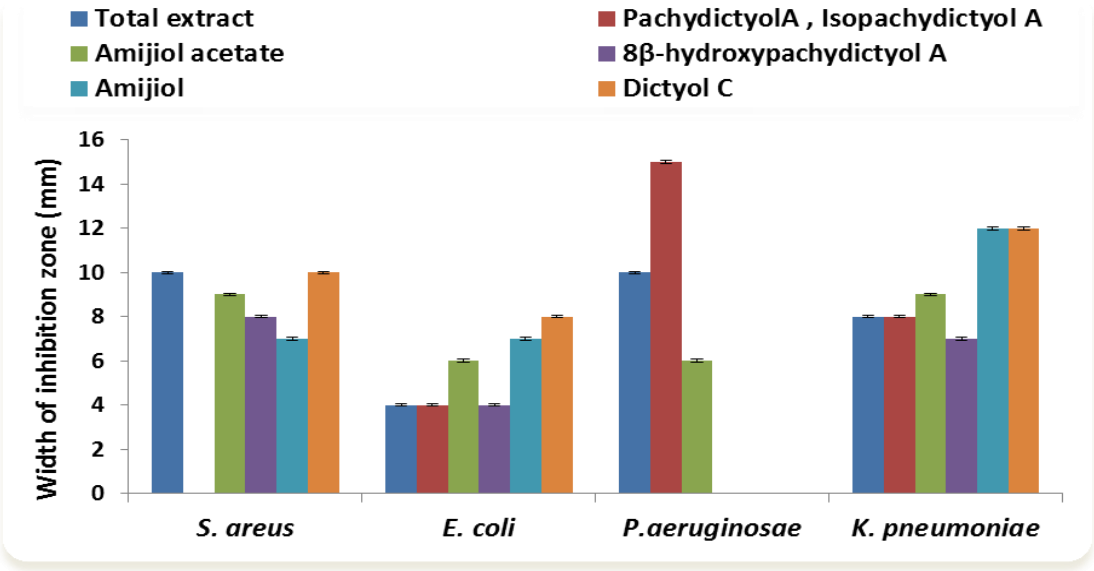
Chloramphenicol	1	0	0	0	0
	10	10±0.01	8±0.02	11±0.06	10±0.03
	25	14±0.06	12±0.01	12±0.06	12±0.01
	50	15±0.06	12±0.03	12±0.01	12±0.06
Penicillin G	1	29±0.35	9±0	27±0.03	0
	10	31±0.35	20±0.06	32±0.06	0
	25	32±0.06	22±0.03	32±0.35	6±0
	50	32±0.03	22±0.06	32±0.06	6±0

Figure 2 showed that the concentration of Amijiol, Amijiol acetate and Dictyol C (50 mg/ml) of *Dictyota dichotoma* ranged between 0 to 12 mm. The highest value (12) mm against *K. pneumonia* and the lowest value (0 mm) were recorded against *P. aeruginosa.* In contrast Abou-El-Wafa*et al.,* (2013) recorded a weak antimicrobial property of dictyol. Pachydictyol A, Isopachydictyol A and total extract showed the highest antibacterial activity 15 mm and 10 mm, respectively against *P. aeruginosa* which is an important and prevalent pathogen among burned patients capable of causing life-threatening illness [14, 25]. It also causes diseases like mastitis, abortion and upper respiratory complications [26]. But they exhibited the same lowest effect against both *E. coli* (4 mm) and *K. pneumonia* (8 mm). On the other hand, 8β-hydroxypachydictyol A exhibited more activity (8 mm) against *S. aureus* and it completely devoid of any activity against *P. aeruginosa*.

Figure 2. Antibacterial activities of different compounds (50 mg/ml) from *D. dichotoma*



As evident in Table 6 extracts of *D. dichotoma* were relatively more effective against the tested fungi. The results were agreeable with the findings reported by Ibraheem *et al.* (2017) and El-Fatimi *et al.* (2013) which concluded that the crude methanolic macroalgal extracts of tested algae *Dictyota linearis* and *Dictyota dichotoma* exerted antifungal activity against different fungal species relatively more effective compared with *Ulva lactuca* [23, 27]. In contrast, Abou-El-Wafa *et al.* (2013) displayed a weak antifungal activity of pachydictyol against *C. albicans*, our results showed that the highest value of all diterpenoids compounds was 20 mm recorded against *Candida albicans* while the lowest value was 1 mm recorded against the others fungal species [14]. All concentrations of **1**, **2** and **3** did not show any noticeable activity against *Penicillium notatum*. Amijiol showed more activity against *C. albicans* (10-16 mm) and *F. oxysporium* (15- 19 mm) while it was completely devoid of any activity against *Aspergillus fumigatus*. The results also showed that the higher concentrations of extract, the greater the effect on the fungal growth inhibition occurred, these findings were in agreement with Ibrahim and Lim, 2015 [28]. The reduction in growth possibly occurred due to interference by active compounds in the extract [29]. Similarly, Lim *et al.* (2011) and Darah *et al.* (2015) reported that the higher concentration of the extract was needed to kill the microorganisms' cells than to inhibit the growth of these cells on time-kill profile study [30, 31].

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Table 6. Antifungal activities of the isolated compounds from *D. dichotoma*

Compound	mg/ml	width of inhibition zone (mm)				
		Asprergillus niger	.A *fumigas*	*Penicillium notatum*	*Fusarium oxysporium*	*Candida albicans*
Total Extract	1	1±o	0	0	1±0	14±0.03
	10	4±0	0	0	8±0.01	16±0.01
	25	8±0.01	4±0	0	10±0.03	17±0.03
	50	9±0	5±0.06	2±0.06	11±0.06	20±0.06
Pachydictyol A, Isopachydictyol A	1	3±0	0	0	6±0.01	0
	10	5±0	0	0	8±0.06	2±0
	25	6±0	0	0	10±0.06	16±0.06
	50	7±0.01	2±0.06	0	12±0.06	17±0.03
Amijiol acetate	1	4±0	1±0	0	5±0.06	1±0
	10	6±0	3±0	0	7±0.06	15±0.06
	25	8±0.01	5±0.	0	9±0.06	16±0.03
	50	9±0.03	5±0.061	0	10±0.06	17±0.03
8β- hydroxypachydictyol A	1	1±0.03	1±0	0	1±0.06	0
	10	2±0	2±0	0	8±0.06	14±0.06
	25	4±0	4±0.01	1±0.06	10±0.06	15±0.35
	50	8±0.01	5±0.01	2±0.06	12±0.06	16±0.06
Amijiol	1	4±0	0	0	10±0.06	15±0.03
	10	6±0.03	0	0	11±0.06	16±0.01
	25	9±0.06	0	0	10±0.06	17±0.35
	50	10±0.06	0	2±0.06	16±0.06	19±0.06
Dictyol C	1	4±0.06	0	0	9±0.06	13±0.03
	10	6±0.01	0	1±0	10±0.06	14±0.06
	25	9±0.03	0	2±0	14±0.01	17±0.03
	50	10±0.06	2±0	3±0	15±0.03	18±0.06

According to the statistical analysis, all tested fungi were significantly affected by concentrations differences (with higher F ratio) more than that of a compound for all fungal species. The main factors (concentrations and compounds) and their interaction on the antifungal activity of *Dictyota dichotoma* extracts showed a very high significant difference ($p<0.05$) (Table 7). As shown in Figure 3, *C. albicans*was the most effective fungal species followed by *F. oxysporium*, *A. niger* and *A. fumigatus*. On the other hand, *P. notatum* showed less effectiveness.

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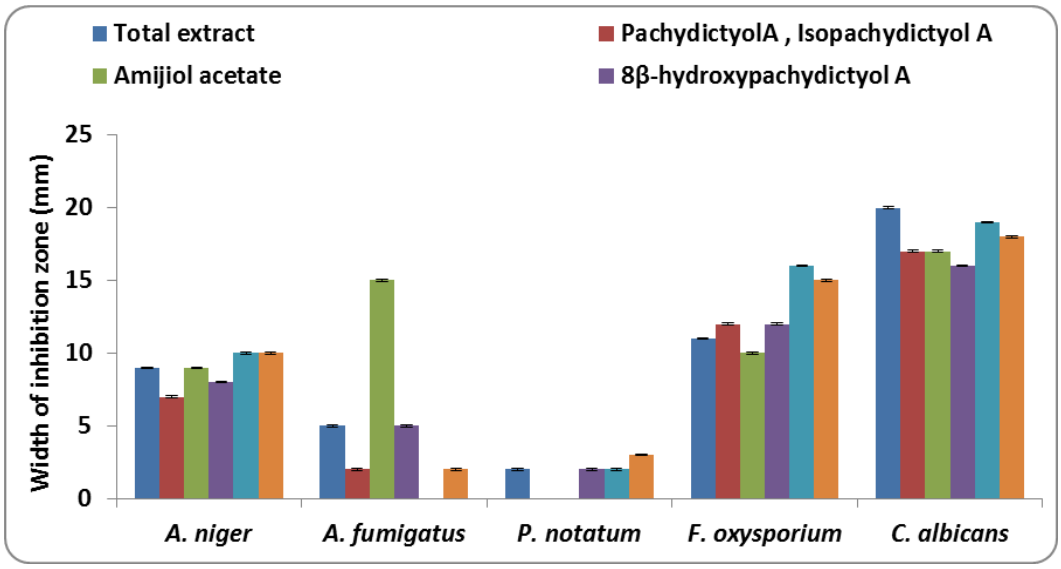
Table 7. Two-way ANOVA showing the effect of the main factors (concentrations and compounds) and their interaction on fungal inhibition zone diameter of *D. dichotoma* extracts

variables and source of variation	*df*	*F*	*p*
Conc	3	80272.3	.000
Fungi	4	272366.4	.000
Compound	5	9165.8	.000
Conc * fungi	12	6383.2	.000
Conc * compound	15	1762	.000
fungi * compound	20	6824.5	.000
Conc * fungi * compound	60	2013.2	.000

Figure 3. Antifungal activities of different compounds (50 mg/ml) from *D. dichotoma*.



As compared with standard drugs, the results revealed that in the extracts for fungal activity, *C. albicans* showed the good results as compared with *P. notatum*. The growth inhibition zone measured ranged from 12 to 24 mm for all the fungal strains. Amijiol effect against *F. oxysporium* was higher than that of miconazole on *A. fumigatus*, *P. notatum*and *F. oxysporium*; this effect increased by increasing the concentration. The total extract exhibited nearly the same effect of griseofulvin on *C. albicans* (Tables 6 and 8). In contrast to El-Fatimi *et al.* (2013) [27], our results showed that *A. niger* was the relatively sensitive fungus while *P. notatum* was the relatively insensitive one.

Table 8. Antifungal activity of standard drugs against fungal test organism

Compound	µg/ml	Width of inhibition zone (mm)				
		Asprergillus niger	A. *fumigatus*	*Penicilliumnotatum*	*Fusarium oxysporium*	*Candida albicans*
Miconazol	1	18±0.06	13±0.06	14±0.03	15±0.06	16±0.06
	10	18±0.06	14±0.06	15±0.03	15±0.06	16±0.06
	25	20±0.06	14±0.15	16±0.03	18±0.06	18±0.06
	50	22±0.06	15±0.06	16±0.03	18±0.06	21±0.06
Griseofulvin	1	19±0.06	18±0.06	12±0.03	16±0.06	18±0.06
	10	23±0.06	18±0.06	13±0.03	18±0.06	21±0.06
	25	25±0.06	22±0.06	17±0.03	20±0.06	22±0.06
	50	28±0.06	23±0.06	17±0.03	20±0.06	24±0.06

Dictyota produces a broad spectrum of chemicals with most being structurally similar prenylated guaiane carbon skeleton diterpenes called dictyols, so this genus has been extensively studied with concerning antifungal secondary metabolites [32-34]. *D. dichotoma* is particularly rich in bioactive terpenes [35]. The

results in Figure 3 indicated that the diterpenoids compounds of algal extracts had active principles that could inhibit the growth of the pathogenic fungi tested except compounds **1**, **2** and **3** did not record antifungal activity against *P. notatum*. However, amijiol and dictyol C showed the lowest value (0 and 2, respectively) against *A. fumigatus*. *D. dichotoma* the crude extract effectiveness had been investigated against *C. albicans* as the highest effective fungi, these results were similar to that of Saleh and Al-Mariri (2018) [36] and in contrast, Manzo *et al* (2009) found that *D. ciliolata* was exhibit moderate antifungal activity against *C. albicans*. This difference may have been due to species [17].

Cytotoxic activity

The data obtained from table 9 showed that total extract displayed cytotoxic effect on both MCF-7 and HepG2 cell lines with IC_{50} 15.3 and 12.9 µg/ml, respectively. Compound **6** came as the most active pure material with IC_{50} 46.2 and 29.3µg/ml against MCF-7 and HepG2 cell lines, respectively. The activity of compound **3** is also appreciated against both cell lines, while the other isolates displayed moderate to weak activities.

Table 9. Cytotoxicity of the total extract and different isolated compounds

	IC_{50}(µg/ml) [a]					
	Total Extract	**1,2**	**3**	**4**	**6**	5-Fu
MCF-7	15.3.0	229.0	113	304	46.2	2.2
HepG2	12.9.0	185.0	95.6	249	29.3	8.2

[a]IC_{50}, (µg/ml): 1-10 (very strong), 11-25 (strong), 26-50 (moderate), 51-100 (weak), 100-200 (very weak), Above 200 (non-cytotoxic).

The free radical scavenging activity of the total extract and the six pure isolated compounds was examined. The best activity was dedicated to the total extract IC_{50}147.6 µg/ml (Vitamin C 14.2 µg/ml). This result in agreement with Habu and Ibeh (2015) which found that comparison of the vitamins showed a higher vitamin C composition [37]. Compound **6** exerted moderate effect with IC_{50} 255.7 µg/ml while the other compounds considered inactive (>1280 µg/ml).

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